Studies in Glycolipids

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Edited by

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PREFACE

Aiming to fill the gap in its field, this book was prepared to be the first with its content. It was aimed to be beneficial not only to academicians and students working in the field of basic science, but also to all clinicians who research the relationship of glycolipids with cancer and immune system diseases. It is thought to be a comprehensive handbook in the fields of Medicine, Biology and Pharmacy, with its content that brings together physicians from different branches and covers up-to-date studies.

Gangliosides, which are the main glycolipids, Ceramides, Glycolipids and protein compounds, which are the main sources in the Glycolipid synthesis step, were selected as other basic topics in the book, which starts with the introduction of glycolipids. The in-vivo and in-vitro display of glycolipids is included in the book. The pharmacological and industrial uses of glycolipids, which have a wide range of uses, are discussed. The book has been developed on the topics of glycolipids and the immune system, sphingolipids and cancer, glycosphingolipid diseases, and glycolipids and infectious diseases to appeal to clinicians. Glycolipids and blood groups and glycolipid degradation products are presented as other study areas of glycolipids. This book will hopefully be useful to all concerned.

—The Editor

CHAPTER ONE

INTRODUCTION TO GLYCOLIPIDS

MUKADDER ERDEM

Introduction

All cells – prokaryote and eukaryote – are surrounded by a plasma membrane that determines the boundaries of the cell and separates its contents from the external environment. Lipids and proteins are the main components of the plasma membrane. The phospholipid bilayer forms the membrane structure by creating a steady barrier between two aqueous compartments, namely the inside and outside of the cell (Figure 1.1).

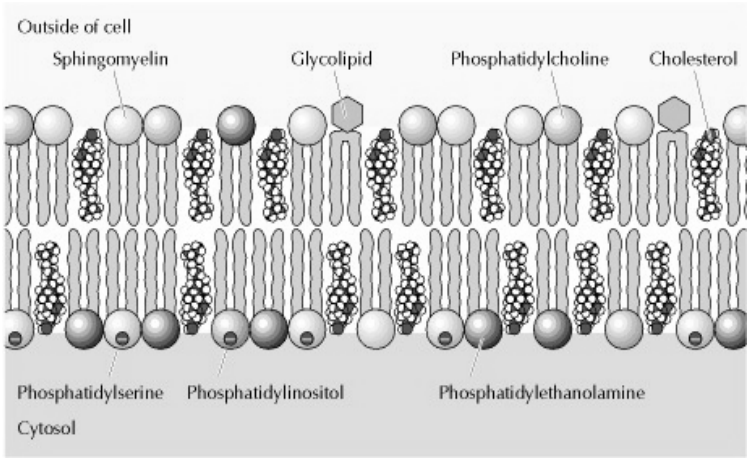

Figure 1.1: The plasma membrane lipid components (Cooper and Hausman 2007)

Mammalian plasma cells contain glycolipids and cholesterol in addition to phospholipids. Glycolipids and their carbohydrate portions are found on the outer surface of the cell membrane. Glycolipids constitute about 2% of membrane lipids (Cooper and Hausman 2007).

Glycolipids are found primarily on the plasma membrane and the body fluids of almost all vertebrate cells. Structural diversity is one of the important features of glycolipids and to date 172 neutral GSLs, 24 sulphated GSLs, and 188 gangliosides have been reported in vertebrates (Yu et al. 2007).

Almost all glycolipids are ceramide derivatives (Figure 1.2) as in sphingomyelin (ceramide: sphingosine + long chain fatty acid). So, referring to them as glycosphingolipids is more precise (Ferrier 2013).

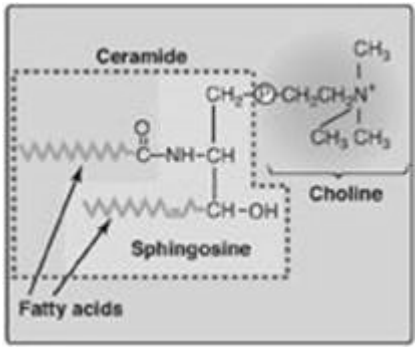

Figure 1.2: Sphingomyelin structure (Ferrier 2013)

Like phospholipids, glycosphingolipids are basic components of all cell membranes, but found in greater amounts in nerve tissue. They interact with the extracellular medium through their localization on the cell membrane. Therefore, they play a role in the regulating of cellular interactions (e.g. adhesion, recognition), growth and development (Ferrier 2013).

Membrane glycosphingolipids have a significant role in the regulating of signal transduction and membrane trafficking. These GSL- and cholesterol-rich laterally assembled micro domains (rafts) act as platforms for the attachment of lipid-modified proteins (e.g. glycosylphosphatidylinositol (GPI)-anchored proteins), so they function in regulating cellular processes.

Glycosphingolipids exhibit antigenic properties and are sources of blood group antigens such as A, B, O and some embryonic and tumor antigens. [The antigenic determinant part is the carbohydrate part of the GSL.] They also act as cell surface receptors for cholera, diphtheria toxins, and some viruses.

Deficient degradation of glycosphingolipids, seen in some genetic disorders, causes lysosomal accumulation of these compounds. Transformed cells with dysregulated growth show typical variances in the carbohydrate portion of the GSL (Ferrier 2013).

Glycolipid Structure

The basic structure of a glycolipid is formed by the attachment of a sphingolipid or a glycerol group to a mono- or oligosaccharide group. The SL or glycerol group can be acetylated or alkylated. So subclasses of glycosphingolipids and glycoglycerolipids are formed (Figure 1.3). Glycolipids interact and attach to the lipid bilayer surface with the hydrophobic structure of the lipid tail (Glycolipids-Physics Libre Texts 2019).

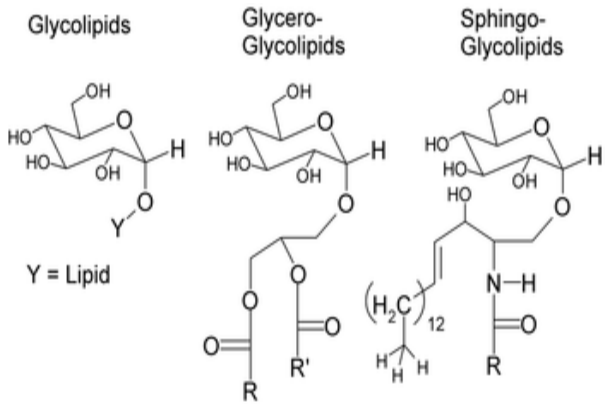

Figure 1.3: Glycolipid structure (Glycolipids-Physics Libre Texts, 2019)

A glycolipid structure consisting of two hydrocarbon chains attached to a polar head group containing carbohydrates is shown in Figure 1.4.

In order to fully understand the glycosphingolipids, we have to talk about sphingolipids. Sphingolipids are composed of a "sphingosine base" (serine + long chain fatty acyl-CoA) (Fahy et al. 2005; Hirabayash et al. 2006). The basic component of these lipids is called a sphingosine.

There are several classes of sphingolipids: the sphingoid base and simple derivatives, ceramides, and complex sphingolipids (Figure 1.5) (Sphingolipid-Physics Libre Texts, 2019).

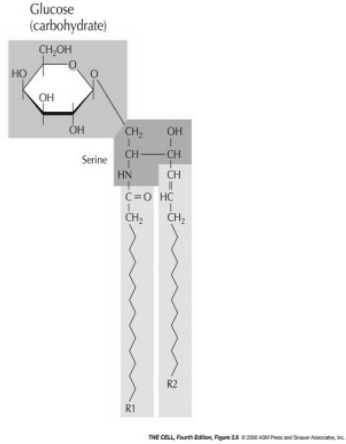

Figure 1.4: A glycolipid structure (two hydrocarbon chains + a polar head group (serine + carbohydrates e.g. glucose)) (Cooper and Hausman 2007)

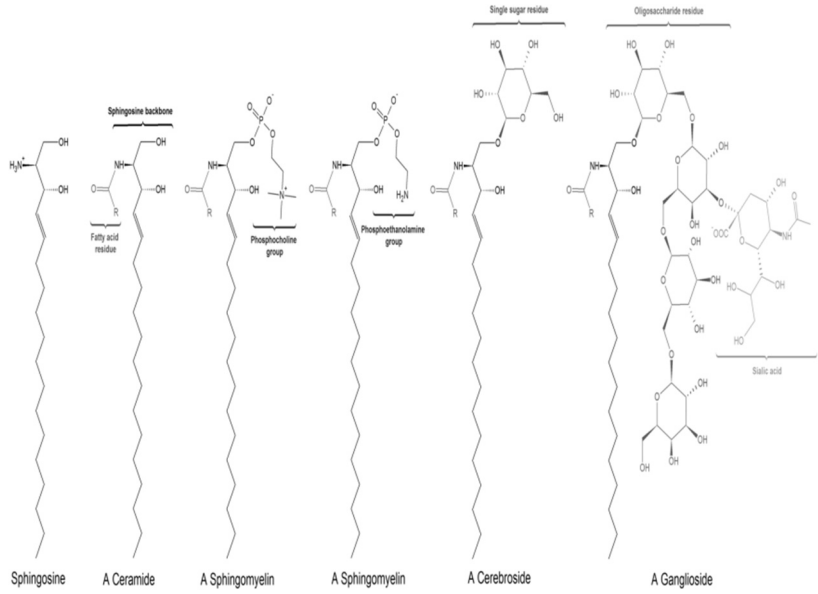

Figure 1.5: Sphingolipid's general structure (Sphingolipid-Physics Libre Texts, 2019)

Synthesis of Glycolipids

Glycolipid synthesis acts through a series of enzymes that sequentially add sugar to the lipid. Lactosylceramide is used to get glycosphingolipids via a series of reactions starting with the acylation and desaturation of D-erythro-sphinganine as a first step. Ceramide is extracellularly glycosylated then β-galactosylated in order to form lactosylceramide. Glycosyltransferases and sulfotransferases can provide further elongation. For example, galactosyltransferases transfer a galactosyl from UDP-Gal onto diacylglycerol for the synthesis of β-galactosyldiacylglycerol in plants. Then an additional transfer of a galactosyl from UDP-Gal causes further elongation (Yu et al. 2010).

Types of Glycolipids

Glycoglycerolipids

These are the glycolipids formed by the binding of diglyceride hydroxyl groups with mono-, di- or tri-saccharides by a glycosidic bond. Monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) are the major glycolipid components.

Monogalactosyldiacylglycerols and digalactosyldiacylglycerols are the main lipids that prevail in chloroplasts of photosynthetic organisms (e.g. plants, algae, bacteria) (Heinz 1996).

Glycosphingolipids

Glycosphingolipids consist of ceramide and one or more monosaccharide residues attached to it by beta glycoside bonds. The ceramide is formed by the binding of a long-chain fatty acid to the -NH2 group of sphingosines by amide bonds (ceramide: sphingosine + fatty acid). Glycosphingolipid subclasses:

Neutral glycosphingolipids; These do not carry any ionic charge. They consist of one or more sugar residues linked by an O-ester bond to the first carbon of ceramides (e.g. cerebrosides). Cerebrosides are ceramide monosaccharides, so they are the simplest neutral glycosphingolipids (e.g. galactosylceramide, glucosylceramide). Galactosylceramides are found in high concentrations in the myelin sheaths of all nerve tissues (central and peripheral). They can constitute 2% and 12% of the dry weight of gray and white matter, respectively (Figure 1.6) (Christie 2003). Glucosylceramide

(Glcß1-1'Cer) is the glycosphingolipid of non-nervous tissues. It is found in small amounts in the brain and some tissues (e.g. spleen, erythrocytes).

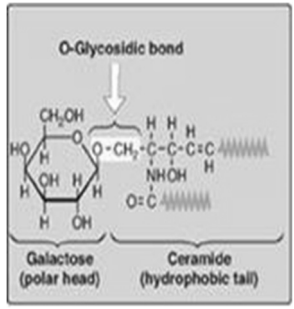

Figure 1.6: Structure of a neutral glycosphingolipid, galactocerebroside (Ferrier 2013)

Oligoglycosylceramides are neutral glycosphingolipids that contain two or more sugar units in their structure. They are found in high numbers in the cell membrane of most eukaryotic organisms (Christie 2003). The most important and abundant oligoglycosylceramide is β-D-galactosyl-(1-4)-β-D-glucosyl-(1-1')-ceramide, also called lactosylceramide (LacCer).

Acidic glycosphingolipids:

These are negatively charged at physiological pH. The negative charge comes from NANA (N-acetylneuraminic acid) in gangliosides (Figure 1.7), and sulphate groups in sulfatides (Ferrier 2013).

Subclasses are as follows:

*Sulfoglycosphingolipids***:** These are also sometimes called sulfatides or sulfatoglycosphingolipids. The 3rd carbon of the sugar residue attached to the ceramide is esterified with sulphate. The main sulfatides are 3-sulfate esters of galactosylcerebrosides. These are found in high amounts in nerve tissue, the myelin sheath and tissues with high sodium transportation such as the kidneys (Ishizuka 1997).

Gangliosides: These are complex glycosphingolipids derived from glucosylceramide containing one or more sialic acid molecules (as shown in Figure 1.7). They are found in high concentrations in the nervous tissue (e.g. up to 6% of the brain lipid weight). GM1 is one of the common

monosialo-gangliosides (G: ganglioside, M: monosialo, 1: migration rate on chromatography).

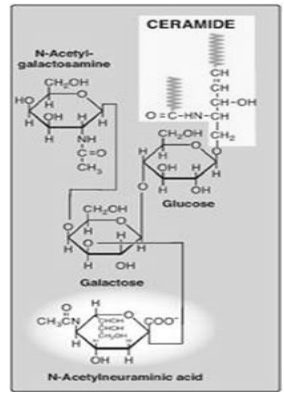

Figure 1.7: Ganglioside GM_2 structure (represents a hydrophobic hydrocarbon chain) (Ferrier 2013)

Glycolipid Distribution in the Cell

Glycolipids are commonly found in the membranes of cells and organelles. The proportion of glycolipids in the membranes of intracellular organelles is about two-thirds of the total cell glycolipid content (Gillard 1993). Glycolipid biosynthesis occurs in the Golgi apparatus. Sugar residues are added to the ceramide individually from the appropriate nucleotide sugar donors. While the first sugar transfer to ceramide occurs on the cytosolic surface of the Golgi complex, other sugar transfers take place on the lumen surface (Edidin 2003). The transportation of most glycolipids between the membranes occurs as small bilayer vesicles.

There is also the cytosolic distribution of some glycolipids. Glycolipids are found on the outer surface of the plasma membrane and on the lumen surface of organelles (Pike 2004). Glycosphingolipid distribution differs on the apical and basolateral sides in the cell, and they are commonly found on the apical side in polarized epithelial cells. Simons and Toomre (Simons and Toomre 2000) observed that cholesterol and glycosphingolipids form glycolipoprotein micro domains called rafts. The lipid content of the plasma membrane and the raft differs. While the raft cholesterol and sphingolipid ratio is 2-3 times higher than that of the cell membrane, the phospholipid content is relatively lower. A very large proportion of cell

glycolipids is packed in a raft. The intensive hydrophobic interactions of lipids in the rafts make them more saturated and denser regions than the other parts of the cell membrane.

Conclusion

Glycolipids undertake many functions in cell such as providing cell membrane stability, cell signal transmission, and intercellular interaction. The interaction of these cell surface markers plays an important role in the regulation of growth and development. Glycolipids are antigenic. The carbohydrate portions of certain glycolipids take part in the determination of human blood groups and safely determine which blood group will be given to which person in blood transfusion. They also act as cell surface receptors for cholera and diphtheria toxins as well as for some viruses. In this sense, more detailed research on glycolipids will be promising for the treatment and prevention of diseases.

Keywords: *Ceramide, ganglioside, glycolipid, glycoglycerolipid, glycosphingolipid, sphingosine*

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CHAPTER TWO

GANGLIOSIDES

GÖKÇE ATİKELER

Introduction

Gangliosides are glycosphingolipids containing sialic acid moieties in their lipophilic ceramide component and a carbohydrate chain. Ernst Klenk, a German scientist, was the first to isolate gangliosides from bovine brain tissue in 1942 (Fishman 1976). Later studies have shown that gangliosides are abundant in the nervous system of vertebrate animals, particularly in gray matter, and in other tissues and organs (liver, kidney) (Kolter 2012). Gangliosides are abundant in the nerve cell membranes of animals (Miller-Podraza et al. 1982).

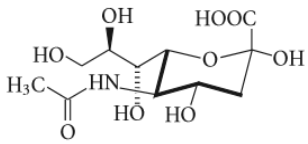
Gangliosides are involved in cell-cell interactions, the binding of bacterial toxins and viruses, and cell communication, which are particularly crucial for brain development and neural differentiation. The content and level of gangliosides change in chronic and neurodegenerative diseases, affecting both disease progression and treatment strategies.

Structure and Functions of Gangliosides

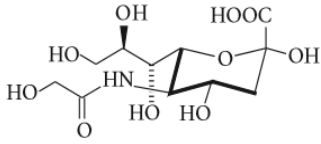
The lipid component of gangliosides, known as ceramide, is composed of long-chain fatty acids linked via an amide bond to amino alcohol (2-amino-1,3-dihydroxy-octadec-4-ene) sphingosine.

The oligosaccharide chain of gangliosides binds via a glycosidic bond to the first carbon atom of the sphingosine (Fishman and Brady 1976). The oligosaccharide component is a combination of glucose, galactose, and N-acetylgalactosamine. Oligosaccharide chains depend on the sugar structure, content, and linkages (Yu et al. 2011).

The negatively charged sialic acid residues separate gangliosides from neutral glycosphingolipids and sulfatides. There are generally 1-4 sialic acid residues, and sometimes seven. Sialic acid is another name for 5-amino-3,5-dideoxy-D-gylcero-D-galacto-non-2-ulopyrasonic acid or neuraminic acid derivatives (Sonnino et al. 2007). The three main sialic acids are 5-N-acetyl-, 5-N-acetyl-9-O-acetyl-, and 5-N-glycolyl (Figure 2.1), the first two of which are found in healthy individuals (Sonnino et al. 2007).



N-acetyl-α-neuraminic acid
(Neu5Ac)



N-glycolyl-α-neuraminic acid
(Neu5Gc)

Figure 2.1: Sialic Acids (Kolter 2012)

Svennerholm was the first to classify gangliosides in 1946. In the classification, the letter "G" indicates the members of the ganglion family, the letter "M" (mono) "D" (di), "T" (tri), or "Q" (tetra) indicates the number of sialic acid residues, and the number 1, 2, or 3 indicates the sequence of migration in thin-layer chromatography. Five subtracted from that number is the number of neutral carbohydrates in gangliosides (Kolter 2012). The galactose to which the sialic acid residues of gangliosides with 0, 1, 2, and 3 sialic acids bind, is referred to as the asialo (0-), a-, b-, and s-series, respectively (Figure 2.2). N-galactosamine to which sialic acid residues bind, is referred to as the α series.

For example, the ganglioside "GQ1b" is of the ganglio-series (G) with four sialic acid residues (Q), four neutral carbohydrate residues (5 – 1 = 4), and two sialic acids bound to the inner galactose (b) (Figure 2.3).

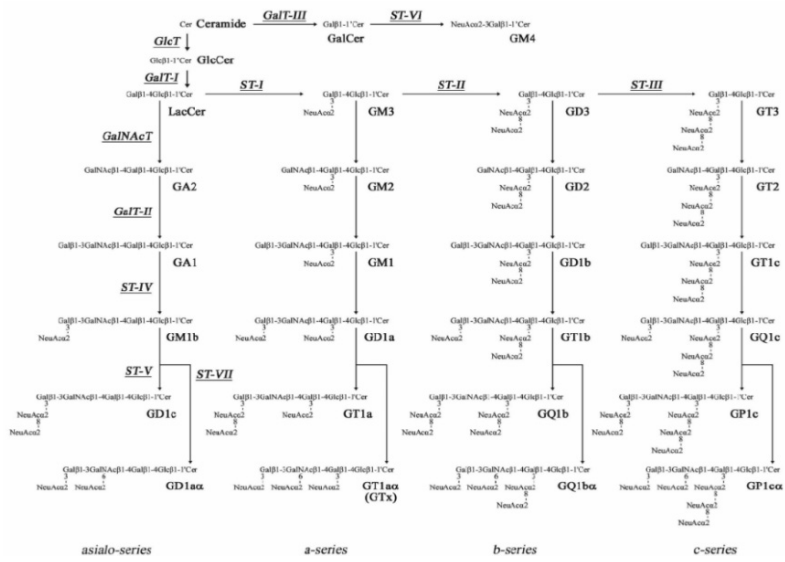


Figure 2.2: Structure and Biosynthetic Pathways of Gangliosides (Yu et al. 2011)

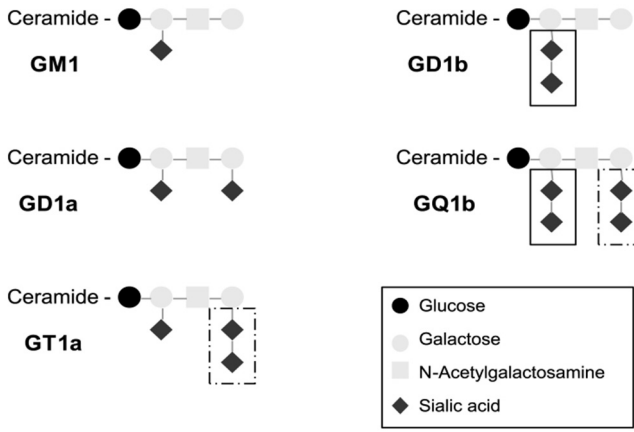


Figure 2.3: Structure of Gangliosides (Delmont and Willison 2015)

KDN (2-keto-3-deoxy-D-glycero-D-galacto-nononic acid) is a recently discovered member of the sialic acid family. The acyl group on the C-5 carbon is replaced by the hydroxyl group (Figure 2.4). KDN was first isolated from rainbow trout eggs (Nadano et al. 1986). Further studies

have found it to be widely distributed in nature, from bacteria to humans (Inoue S., Kitajima K., and Inoue Y. 1996). KDN is synthesized de novo from mannose (Angata et al. 1999). KDN synthesis requires CMP-KDN synthase and KDN-transferase enzymes. KDN expression is age-dependent. In rat liver, KDN decreases from birth to adulthood and then increases again with age (Campanero-Rhodes et al. 1999). The ratio of free KDN/free Neu5Ac in patients with ovarian cancer is positively correlated with the cancer stage, suggesting that KDN can be used as a biomarker for ovarian cancer (Inoue et al. 1998). High levels of KDN observed in ovarian cancer patients have facilitated research on other types of cancer.

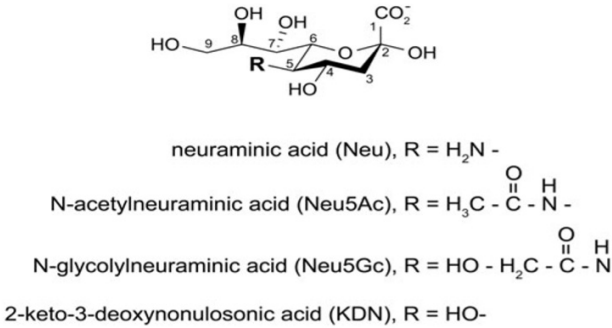


Figure 2.4: Chemical Structure of KDN (Wang et al. 2015)

Occurrence of Gangliosides

Most gangliosides in adult mammals belong to the ganglio, gala, and lacto series. Predominantly found in the brain, gangliosides are five times greater in gray matter than in white matter (Kolter 2012). Gangliosides are 6-10% of the lipid in the brain. Ganglioside production in the brain is proportional to neurogenesis, synaptogenesis, and cell proliferation (Rahmann 1995). The main gangliosides in the brain are GM1, GD1a, GD1b, and GQ1b. Simple gangliosides (GM3 and GD3) turn into complex gangliosides (GD1a and GT1b) during brain development (Yu et al. 2009). The content and structure of gangliosides in the brain change with age. Lipid-bound sialic acid concentrations decrease, while the number of gangliosides with complex carbohydrates increases.

Extraneuronal tissues (liver, bone marrow, kidney, and embryonic stem cells) have ganglio and lactosyl series. The structure and composition of gangliosides in the liver also change with age (Ozkok et al. 1999)

The serum also contains GM3, GD3, GD1a, GM2, GT1b, GD1b, and GQ1b, which are mainly transported by LDL (66%), HDL (25%), and VLDL (7%) (Senn et al. 1989).

Gangliosides are found in numerous vertebrate cells. At the cellular level, they are mostly found in the plasma membrane. The mitochondrial membrane has GD3 that regulates apoptosis (Garofalo et al. 2007). The nucleus membrane also has GD3 that helps to stabilize calcium (Ledeen and Wu 2011).

Gangliosides with O-Acetylated sialic acids are found mainly in growing cells and tissues. They are used as oncofetal markers in different tumors (Kohla et al. 2002) and may act as receptors for coronaviruses (Schwegmann and Herrler 2006).

Gangliosides are also found in different digestible foods (meat, milk, eggs, etc.). Milk contains GD3 and GM3 (McJarrow et al. 2009). Gangliosides in foods help to regulate intestinal microflora and prevent infections, especially in neonates. A considerable amount (80%) of gangliosides in foods is absorbed in the intestines, resulting in high levels of gangliosides in the serum, which is vital for brain development in neonates (McJarrow et al. 2009).

Biosynthesis of Gangliosides

The first stage of ganglioside synthesis is the formation of ceramide in the endoplasmic reticulum. L-serine and palmitoyl-CoA are catalyzed by the pyridoxal phosphate-dependent serine palmitoyltransferase, resulting in ceramides by the acylation of sphingosine by N-acyltransferases, a member of the LASS family of enzymes (Thomas Kolter 2012). Ceramides are transported to the Golgi apparatus via vesicle transport or ceramide transfer protein (CERT) (Yamaji and Hanada 2015) (Figure 2.5). UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine, and CMP-N-acetylneuraminic acid are used as carbohydrate donors, which are added to ceramides by glycosyltransferase. The resulting glycosylceramides are then converted into LacCer by lactosylceramide synthase (Nishie et al. 2010). LacCer is the precursor of different glycosphingolipid series

(ganglio-, asialo ganglio-, globo-, and lacto-) (Sandhoff and Sandhoff 2018), which are generated by cell-specific glycosyltransferases.

While GM4 is derived from galactosylceramide (GalCer), many other gangliosides are synthesized from lactosylceramide (LacCer). First, GM3 (a simple ganglioside) is a result of sialic acid being added to LacCer by the LacCer α2-3 sialyltransferase (ST-I or GM3 synthase) enzyme. GD3 and GT3 are a result of sialic acid being added to GM3 and GD3, respectively, by the GM3 α2-8 sialyltransferase (STII or GD3 synthase) and GD3 α2-8 sialyltransferase (STIII or GT3 synthase) enzymes. GM3, GD3, and GT3 are the precursors of numerous a-b-c series gangliosides. Sialyltransferase enzymes result in more complex gangliosides (Figure 2.2). Asialo-series gangliosides are synthesized from LacCer by glycosyltransferases along a different pathway (Yu et al. 2011).

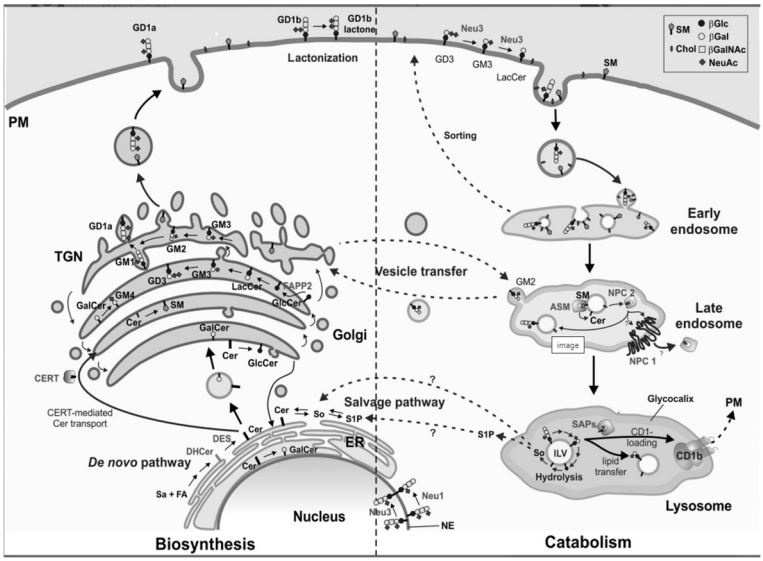


Figure 2.5: Ganglioside Biosynthesis and Catabolism (Sandhoff and Sandhoff 2018)

Degradation of Gangliosides

Gangliosides are degraded mainly in intraendolysosomal vesicles, endosomes, and lysosomes. However, they are also degraded by plasma membrane-associated sialidase (Neu3) (Kolter 2012). Luminal vesicles are the result of vesicle budding and fusion by endosomal complex proteins. Vesicles

are removed for lysosomal digestion through lipid sorting at the endosome stage (Wollert and Hurley 2010). Cholesterol is sorted out by two sterol binding proteins (NPC-1, NPC-2) (Abdul-Hammed et al. 2010) and degraded in sphingomyelin by sphingomyelinase. Anionic bis(monoacylglycero)phosphate (BMP) stimulates ganglioside degradation and is derived from phosphatidylglycerol in intraendosomal membranes (Gallala and Sandhoff 2011). Although the lysosomal perimeter membrane is resistant to lysosomal digestion, intra-endosomal membranes are degraded by sphingolipid activator protein (SAP) and hydrolytic enzymes (Sandhoff and Harzer 2013). The sialic acid and carbohydrate residues of gangliosides are removed by sialidase and exoglycohydrolase, respectively, while the resulting ceramides are hydrolyzed into fatty acids by ceramidases (Sandhoff and Sandhoff 2018). This degradation that occurs through the endocytosis-endosome-lysosome pathway requires acidic pH. On the other hand, sialidases and glycohydrolases require effector molecules, referred to as sphingolipid activator proteins (SAPs) (Kolter and Sandhoff 2005). Hereditary defects in prosaposin – the precursor of saposin A, B, C, and D – result in the deposition of glycosphingolipids and gangliosides (Sandhoff et al. 2018).

The first stage of the degradation of complex gangliosides in mammalian tissues is the removal of terminal sialic acid from oligosaccharide chains by neuraminidases and the formation of GM1 (Sandhoff and Sandhoff 2018). The degradation of GM1 proceeds with the formation of GM2 by the removal of galactose by the GM1-β-galactosidase enzyme (promoted by the GM2 activator protein or saposin B). Afterward, GM3 is formed by the removal of terminal N-acetylgalactosamine residues by the β-hexosaminidase A enzyme (promoted by the GM2 activator protein). GM3 is degraded to LacCer by α-sialidase and SAP B. LacCer is degraded to GlcCer by the β-galactosidase enzyme with the help of SAPs B and C. Glycosyl residues are also removed with the help of SAP B and C galactosidase. The resulting ceramide is degraded to sphingosine and free fatty acids, respectively, by ceramidase and SAP D (Figure 2.6). Hydrolytic stages in GM1 degradation are affected by pH, positively charged molecules, negatively charged molecules, and SAP. For example, GM2 is degraded by Hex A and GM2AP at $3.8<pH<4.5$ (Bierfreund et al. 1999).

Ganglioside degradation involves not only SAPs, but also anionic lipids, the absence of which makes the process difficult (BMP, phosphatidic acid, phosphatidylglycerol, and phosphatidylinositol) (Sandhoff R., Schulze H. and Sandhoff K. 2018). Anionic lipids stimulate, whereas cationic

lipids inhibit degradation (sphingosine bases). The terminal degradation product of lysosomal sphingolipids is sphingosines, which should be effectively removed from the lysosome for successful degradation. Ceramides stimulate glycosylceramide hydrolysis in the presence of fatty acids, monoacylglycerol, diacylglycerol, and anionic lipids, whereas sphingomyelin inhibits the hydrolysis of sphingosine and results in the deposition of glycosylceramide (Sandhoff et al. 2018).

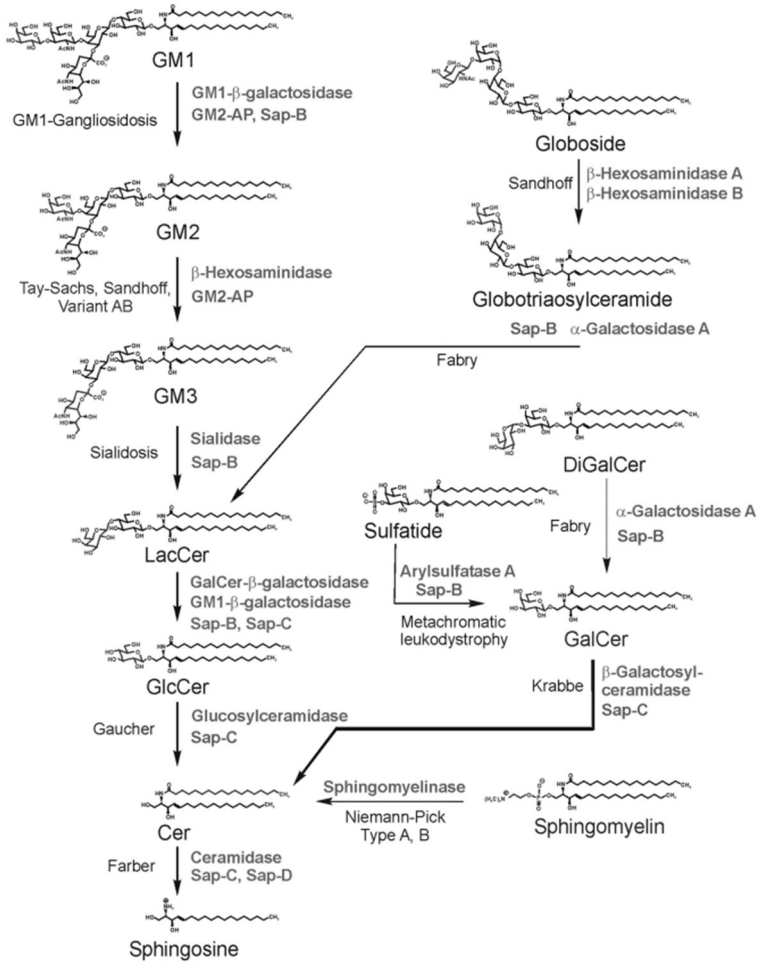


Figure 2.6: Ganglioside Degradation and Gangliosidoses (Sandhoff and Kolter 1995)

Gangliosidoses

GM1 and GM2 gangliosidoses are lysosomal storage diseases associated with ganglioside degradation defects. Gangliosides are found in the neuronal plasma membrane, and defects in ganglioside metabolism can cause fetal neurodegenerative diseases, such as GM1 and GM2 gangliosidoses due to defects in lysosomal ganglioside degradation (Sandhoff and Harzer 2013). All gangliosidoses are inherited autosomal recessive diseases with severe variable symptoms that can occur at any age as a result of genetic defects in ganglioside degradation.

GM1 gangliosidosis is a hereditary disease characterized by GM-1-β-galactosidase deficiency in lysosomes. GM1 gangliosidosis is caused by defects in the GLB1 gene and characterized by the deposition of GM1 and GA1 in neuronal cells (Brunetti-Pierri and Scaglia 2008). GM1 gangliosidosis presents in three clinical forms. Infantile (type 1) is characterized by the progressive deterioration of the nervous system. Its symptoms begin to appear in the neonatal period with a life expectancy of about two years. The other two types are juvenile (type 2) and adult/chronic (type 3) (Sperb et al. 2012). The severity and progression of the disease are proportional to the residual enzyme activity in cells. B-galactosidase is specific for oligosaccharide and keratan sulphate, and therefore, oligosaccharidosis and mucopolysaccharidosis findings can be regarded as extraneuronal clinical findings in the absence of B-galactosidase (Suzuki and Namba 2001).

The infantile form usually occurs in the first six months of life. Individuals with the infantile form usually seem normal until symptoms appear, but their development slows down over time, and they begin to suffer from muscle atrophy. Individuals with the infantile form lose their skills over time and have loud and exaggerated startle reflexes. They show common symptoms of hepatosplenomegaly, skeletal abnormalities, seizures, reduced mental capacity, corneal clouding, a cherry-red spot in the macula of the eye, and cardiomyopathy due to gingival hypertrophy and heart muscle weakness.

The juvenile form (type 2) is an intermediate form. Individuals with the juvenile form have normal early development. They usually begin to show symptoms between the ages of 18 months and five years. It is characterized by developmental retardation but not by a typical facial appearance, a red spot in the macula of the eye, and organomegaly. The

juvenile form progresses more slowly than the infantile form, but the life expectancy is as short.

Individuals with the adult form (type 3) show symptoms in adulthood. The characteristic findings are dystonia and vertebral abnormalities. Life expectancy varies.

GM2 gangliosidosis is characterized by a GM2 ganglioside degradation defect. It manifests itself in three forms depending on hexosaminidase isoenzymes (Kolter 2012). Variant B, known as Tay-Sachs disease, affects hexosaminidase A and S but not hexosaminidase B. Variant O, known as Sandhoff disease, is characterized by a deficiency of beta-hexosaminidase A and B and a normal S activity. Variant AB is characterized by normal hexosaminidase A, B, and S activities, but a deficiency in GM2 activator protein due to mutations in the GM2 activator gene.

Tay-Sachs disease has three forms; infantile, juvenile, and adult/chronic. Individuals with the infantile form are normal at birth. However, the infantile form is characterized by progressive motor weakness, a cherry-red spot in the macula of the eye, increased startle responses at about 3 and 6 months, and progressive muscular atrophy, resulting in hypotonia and death within the first few years of life. The juvenile and adult forms are characterized by increased variant hexosaminidase A activity (Leinekugel et al. 1992) with very heterogeneous symptoms.

Sandhoff disease is characterized by GA2 accumulation in the brain and visceral organs as well as organomegaly and skeletal malformations, similar to the infantile form of Tay-Sachs disease. The juvenile form is also characterized by dementia and cerebellar ataxia with mental retardation.

Variant AB is characterized by GM2 and GA2 accumulation. It is similar to Tay-Sachs disease, but symptoms appear later (Kolter 2012).

As with sphingolipidosis, lysolipid (lysoGM2) increases, and can therefore, be used as a biomarker for Tay-Sachs and Sandhoff diseases (Kodama et al. 2011).

Analysis of Gangliosides

Chemical analysis was used to measure the ganglioside structure and levels, but today they can be measured in lipidomics in mass spectrometry (Farwanah and Kolter 2012). Mass spectrometry is the most widely used

method due to its sensitivity, accuracy, and high speed of analysis. Conventional methods involve a series of extraction and preparation steps (Merrill et al. 2005). Numerous methods can be used for purification (column chromatography and solid-phase extraction) (Muthing 2000). Gangliosides are extracted from tissue and body fluids using chloroform-methanol chemicals. Water in the extract solvent can help to improve the efficiency of extraction (Byrne et al. 1985).

Gangliosides can be classified according to their glycan level using thin-layer chromatography (TLC), HPLC, and mass spectrometry combined with other methods, facilitating the sorting of gangliosides by mass spectrometry (Sisu et al. 2011).

Various protocols have been developed for the mass spectrometry analysis of gangliosides. The ionization technique of biological material depends largely on electrospray ionization mass spectrometry (ESI-MS). However, matrix-assisted laser desorption/ionization (MALDI) is also used (Thomas Kolter 2012). ESI-MS technology can also be combined with liquid chromatography (LC/ESI-MS) (Spiro et al. 2020).

Functions of Gangliosides

Gangliosides perform numerous functions either by interacting with extracellular membrane-bound molecules (trans interactions) or by changing the properties of proteins in the same membrane (cis interactions) (Todeschini and Hakomori 2008). Trans interactions take place between the glycan part of gangliosides on the one side and lectins on the other side.

There are trans interactions between gangliosides and myelin-associated glycoprotein (MAG) in the nervous system. MAG recognizes NeuAcα2-3Galβ1-3GalNAc-termini (Schnaar 2010). It is necessary for miyelin stability and axon regeneration (Schnaar 2010). Cis interactions can be direct or indirect. Gangliosides can affect the activity of tyrosine kinase receptors in the plasma membrane. Thus, epidermal growth factor utilizing tyrosine kinase can also affect the functions of such molecules as insulin (Inokuchi and Kabayama 2007). GM3 binds to the extracellular domain of epidermal growth factor receptors and inhibits tyrosine kinase activity (Kim et al. 2020). GM3 inhibits EGFR activity in various cell cultures (Meuillet et al. 2000). It also inhibits insulin receptor signaling (Kim et al. 2020).

Cis and trans interactions of gangliosides are also crucial for immunity and infectious diseases (Hanada 2005). They can act as coreceptors for viruses, bacteria, and microbial toxins (Neu et al. 2011). The most obvious example is that GM1 acts as a receptor for cholera toxin (S'anchez and Holmgren 2011). Many pathogens use sialic acids in cell-surface glucoconjugates to gain access to the cell. Merkel cell polyomavirus, rotaviruses, and adenoviruses use GT1b (Erickson et al. 2009), sialic acid on GM1 (Haselhorst et al. 2009), and GD1a (Nilsson et al. 2011) as cell receptors, respectively. Due to the direct interactions between lipopolysaccharides and gangliosides, gram-negative bacteria use gangliosides to gain access to the cell (Day et al. 2015). The effects of gangliosides in the immune system (cell activation, signal transmission, cell interaction, etc.) vary from cell to cell. They are found in hematopoietic stem cells, mast cells, granulocytes (GM1), monocytes and macrophages (GM3), B lymphocytes (GM3), and T lymphocytes (GM1 and GM3) (Zhang et al. 2019). GM1 in T and B lymphocytes is particularly essential for the activation of these cells. Brain-derived gangliosides inhibit T cell proliferation (Chu and Sharom 1995) by binding to IL-2 receptors (Lu and Sharom 1995) or IL-4 and IL-5. Monocytes reduce the expression of MHC II antigens (Heitger and Ladisch 1996). NFkappaB inhibits the signal pathway (Caldwell et al. 2003). GM2 and GM3 inhibit NK cell activity (Grayson and Ladisch 1992).

Gangliosides are also involved in cell recognition, adhesion, and signal transmission on the cell surface (Yu et al. 2011). As stated earlier, gangliosides are found in the nuclear membrane as well as the plasma membrane and play a vital role in both cellular and nuclear calcium transport (Leeden and Wu 2008).

Gangliosides reduce the deposition of lipid peroxidation products in rat myocardiocytes (Maulik et al. 1993) and brain cells and increase the removal of free radicals (Avrova et al. 2002). Exogenous gangliosides affect cell functions and protect the cell against oxidative stress (Gong et al. 2018). The antioxidant effects of gangliosides can protect the sperm, oocyte, and embryo from reactive oxygen products (Kim et al. 2020). GT1b shows antioxidant effects by scavenging free radicals, while GM3 induces apoptosis in the early embryo period (Kim et al. 2020). Exogenous gangliosides promote oocyte maturity and pre-implantation embryonic development (Kim et al. 2020).

The biological importance and functions of gangliosides are observed in laboratory animals with ganglioside synthesis defects (Table 2.1). The selective degeneration of the organ of Corti results in hearing loss in ST-I knockout mice (Yoshikawa et al. 2009). Recent research has also reported attention-deficit hyperactivity disorder in ST-I knockout mice, suggesting that glycosphingolipids are involved in maintaining neuropsychological balance (Niimi et al. 2011).

Deficiency in -b and -c series gangliosides and impairment in the regeneration capacity of the damaged hypoglossal nerves together with intact neural tissue are observed in ST-II knockout mice (Okada et al. 2002).

Deficiency in the GalNacT gene results in the reduced GM1, GD1a, GD1b, and GT1b content in the brain. With time, animals exhibit marked impairment in motor coordination, axonal degeneration in sciatic nerves, and demyelination of optic nerves (Sugiura et al. 2005).

GalNacT and STII knockout mice exhibit GM3 ganglioside synthesis mainly in non-cerebral tissues, resulting in weight loss, progressive motor and sensory neuropathy, and impaired learning and memory over time (Tajima et al. 2009).

Ganglioside-deficient mice exhibit marked vacuolation in the cerebellum and white matter as well as cell apoptosis and axonal degeneration (Yamashita et al. 2005).

Gangliosides and Diseases

Gangliosides take place in the pathogenesis of some immune-mediated neurological diseases (e.g., Guillain-Barré syndrome). The pathophysiology of neurological symptoms in Guillain-Barré syndrome presents lipooligosaccharides in the cell membrane of Campylobacter jejuni, similar to gangliosides in nerves. Anti-ganglioside antibodies resulting from molecular similarity are the cause of neurological symptoms (acute polyradiculoneuropathy and acute quadriplegia) in Guillain-Barré syndrome (Kaida et al. 2009). GM3 is involved in the pathogenesis of insulin resistance and type 2 diabetes (Duncan et al. 2018). GM3 is synthesized by GM3 synthase. Diabetic mice exhibit high levels of GM3 and GM3 synthase in the kidneys, liver, fat, and muscle tissue (Tagami et al. 2002). Diabetic patients with microvascular complications also have high serum levels of gangliosides. GM3 synthase suppression in diabetic